REMARKS

Claims 1, 3, 5-12, 14-17 are pending in the application. Claims 1 and 8 have been amended and claims 3, 6, 7, 9, 10, and 12-17 have been without prejudice or disclaimer. Accordingly, claims 1, 5, 8 and 11 will be pending in the application upon entry of the claim amendments and cancellation presented herein.

The claims have been amended to claim more fully the instant invention. Support for the amendments can be found throughout the specification and claims as originally filed. Specifically, claim 1 has been amended to incorporate the recitations of claims 3, 6, 7 9 and 10 and, consdequently, claims 3, 6, 7, 9 and 10 have been cancelled without prejudice or disclaimer. Claim 8 has been amended to correct the dependency resulting from the cancellation of claim 7.

Support for the amendments to claim 1 can be found at least, for example, in the specification at page 37, lines 10 to 14 (Example 5), and in claims 3, 6, 7, 9 and 10 as originally filed. No new matter has been added.

Amendment and cancellation of the claims herein are not to be construed as acquiescence to any objections/rejections set forth in the instant Office Action and/or any previous Office Action and were done solely to expedite prosecution of the application. Applicants reserve the right to pursue the subject matter of the claims as originally filed in this or one or more subsequent patent applications.

Claim Rejections – 35 U.S.C. §112

Claims 12 and 14-17 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. Applicants respectfully disagree and traverse the rejection. However, without acquiescing in any way to the rejection and in order to expedite prosecution, Applicants have cancelled claims 12 and 14-17 without prejudice or disclaimer, thereby rendering the rejection moot.

Claim Rejections – 35 U.S.C. §102

Claims 12 and 14-17 are rejected under 35 U.S.C. §102(b) as anticipated in view of U.S. Patent Publication No. 2003/0044869 to Yeung *et al.* ("Yeung"). Applicants respectfully disagree and traverse the rejection. However, without acquiescing in any way to the rejection and in order to expedite prosecution, Applicants have cancelled claims 12 and 14-17 without prejudice or disclaimer, thereby rendering the rejection moot.

Claim Rejections – 35 U.S.C. §103

Claims 1, 3, 5, and 6-8 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over U.S. Patent No. 4,658,022 to Knowles and Marchesi ("Knowles *et al.*") in view of Macri *et al.*, *Electrophoresis*, 2000, 21: 1685-1693 ("Macri"). Applicants have cancelled claims 3, 6, and 7 without prejudice or disclaimer, thereby rendering the rejection moot as to those claims. Regarding claims 1, 5 and 8, Applicants respectfully disagree and traverse the rejection.

In order to make out a *prima facie* showing of obviousness, there must be some motivation to combine the references, the combination of references must teach or suggest each and every element of the claimed invention, and there must be some reasonable expectation of success in making and using the invention as claimed. (MPEP §2141). In the present case, the references cited by the Office fail to teach or suggest all of the claim limitations; fail to provide the requisite motivation to combine; and fail to provide a reasonable expectation of success.

As noted above, claim 1 has been amended to include the recitations of claims 3, 6, 7, 9 and 10. Thus, as presented herein, claim 1 provides an immunoassay in which the protein solution of step (1) contains a reducing agent and an ionic surfactant and is boiled at least at 80℃ for 5 minutes. That is, the boiling step is conducted in the presence of a reducing agent and an ionic surfactant, and is conducted ***prior*** to the immunoreaction of step (5).

The combination of Knowles *et al.* and Macri does not teach or suggest all the features of claim 1 presented herein. Specifically, Knowles *et al.* in view of Macri does not teach a boiling step and the Office Action, on page 10, admits as much: "However, Knowles in view of Macri fails to teach a method, further including an additional step of a boiling step."

Thus, the cited combination of references does not teach or suggest each and every element of the claim. More specifically, the cited combination of references fails to disclose a boiling step prior to the immunoreaction. Accordingly, at least for this reason, Applicants respectfully request reconsideration and withdrawal of the rejection of Claims 1, 5, and 8 under 35 U.S.C. §103(a).

The Office has rejected claims 9 and 10 under 35 U.S.C. §103(a) as allegedly unpatentable over Knowles *et al.* in view of Macri and U.S. Patent No. 5,645,838 to Winkler *et al.* ("Winkler"). Applicants have cancelled claims 9 and 10 without prejudice or disclaimer, thereby rendering the rejection moot as to those claims. Nevertheless, because claim 1, as currently amended, incorporates the features of claims 9 and 10, Applicants assume that claim 1, as currently amended, would also be subject to the same rejection. Applicants respectfully disagree and traverse the rejection.

As indicated in the preceding arguments and as admitted in the Office Action at page 10, the combination of Knowles *et al.* and Macri fails to teach a method including an additional boiling step. However, the Office Action, at page 10, has further cited Winkler as an alleged remedy for the deficiency of Knowles *et al.* in view of Macri. In this respect, the Office states that it would be obvious to include the boiling step taught be Winkler with the methods of Knowles. Applicants respectfully disagree.

Winkler does not teach that the boiling step can be performed ***prior*** to the immunoreaction. At best, the reference only teaches that the boiling step may be performed prior to analysis by SDS-PAGE electrophoresis, as can be seen from a careful review of Example 6 of Winkler.

In Example 6, the membrane enriched fraction is solubilized in a buffer containing Tris-HCl, NaCl, EDTA, PMSF (serine protease inhibitor), iodoacetamide and

NP-40 (non-ionic surfactant). The solubilized antigen is labeled with ^{125}I and reacted with normal human serum and subsequently with the test serum to form a labeled antigen (Gp 60/50)-antibody (test serum) complex, in which the immunoreaction is carried out at 4℃ or on ice. Then, the immunocomplex is subjected to a protein-A Sepharose CL-4B treatment carried out at 4℃, and the antigen (Gp 60/50)-antibody (test seruin)-Sepharose complex thus formed is isolated and then boiled in a SDS-loading buffer to solubilize the antigen from the antigen-antibody-Sepharose complex. The solubilized antigen is finally subjected to SDS-PAGE electrophoresis.

In contrast to the invention being claimed, Winkler does not boil the antigen solution ***prior*** to the immunoreaction. Nowhere in Winkler is there a teaching or suggestion to heat the antigen solution used in the immunoreactions. In fact, it appears that Winkler avoids heating the antigen solution when the immunoreactions are conducted. In this regard, one of ordinary skill in the art would view Winkler as teaching away from the claimed invention such that there would be no expectation of success in making and using the claimed invention. Moreover, the protein solution of Winkler does not contain a reducing agent. Accordingly, in view of the teachings of Winkler, as construed by one of ordinary skill in the art, one of ordinary skill in the art would have no motivation to boil the antigen solution ***prior*** to the immunoreaction, as presently claimed.

In contrast, the present application demonstrates that a boiling step ***before*** conducting the immunoreaction significantly improves the stability and sensitivity of the immunoassay of the invention. The boiling step is conducted prior to the immunoreaction of a solubilized antigen and an antibody raised against the antigen denatured by using the same reducing agent and ionic surfactant as used in the solubilization of the antigen. These advantageous effects provided by the boiling step are at least described and demonstrated in Example 6 of the filed specification"(2) Effect of boiling of a sample solution" (from page 41, line 6 to page 42, line 22, and Figs. 15 to 22). There is no teaching or suggestion in any of the cited references, taken alone

or in any combination, that boiling prior to the immunoreaction step would afford these advantageous effects.

Accordingly, based on Winkler, one of ordinary skill in the art would not be motivated to include the boiling step prior to the immunoreaction as presently claimed. Winkler only teaches the boiling step immediately before analysis by SDS-polyacrylamide gel electrophoresis, which is subsequent to performing the immunoreaction. Winkler is otherwise silent about boiling the protein solution prior to the immunoreaction. Moreover, without the benefit of the disclosure of the present application, the advantageous effects of the boiling step prior to the immunoreaction (*e.g.*, as shown in Figs. 15 to 22 of the filed specification), would not have predictably resulted by combining the teachings of the cited references. Because the cited combination of references does not put one of ordinary skill in the art in possession of the claimed invention, one of ordinary skill in the art would not have a reasonable expectation of success in making and using the claimed invention.

In sum, the methods of the invention are not obvious, at least because the cited references do not teach or suggest boiling the protein solution prior to the immunoreaction. The teachings of the cited references either alone or in combination would not put one possession of an immunoassay wherein the protein solution is boiled prior to the immunoreaction. Therefore Applicants respectfully request withdrawal of the rejections of claims 1, 5, and 8 under 35 U.S.C. §103(a).

Claim 11 is rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Knowles *et al.* in view of Macri and Yeung. Applicants respectfully disagree and traverse the rejection.

Claim 11 depends from claim 1 and, therefore, recites a boiling step ***prior*** to the immunoreaction step. As indicated in the above response to the rejection of claims 1, 5, and 8 under 35 U.S.C. §103(a), Knowles *et al.* and Macri do not teach or suggest boiling the protein solution prior to the immunoreaction. Likewise, Yeung also does not teach or suggest boiling the protein solution prior to the immunoreaction and, therefore, does not

make up for the deficiencies in Knowles *et al.* and Macri. Accordingly, the cited combination does not teach or suggest each and every element of the claimed invention.

In summary, Applicants respectfully argue that the claimed invention of U.S. Patent Application No. 10/501,110 is non-obvious in view of all of the cited references individually or in combination. Accordingly, Applicants respectfully request that the Office reconsider and withdraw the foregoing rejections.

CONCLUSION

In view of the foregoing amendments and arguments, Applicants respectfully request reconsideration and withdrawal of all pending objections/rejections and allowance of the applications with claims 1, 5, 8, and 11 presented herein. If a telephone call with Applicants' representative would be helpful in expediting prosecution of the application, Applicants invite the Examiner to contact the undersigned at the telephone number shown below.

Applicants believe that no fees are required for consideration and entry of this paper. Nevertheless, the Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. **04-1105**, under Order No. 61625(70232).

Dated: August 11, 2009

Respectfully submitted,

Electronic signature: /Elbert Chiang, Ph.D./
Elbert Chiang, Ph.D.
Registration No.: 60,325
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5502
Attorneys/Agents For Applicants